Appendix C

Approach for Estimating the Magnitude of Chronic Effects of Cyanide on Listed Species

The first preference for quantitatively assessing chronic effects of cyanide on listed species would be to use data from chronic toxicity tests/studies with the species in question. If, however, chronic toxicity data are not available or are not suitable it may be necessary to estimate effects on listed species using surrogate species. For cyanide, species-specific chronic toxicity data are not available for the listed species for which "likely to adversely effect" (LAA) determinations have been made. To estimate the type and magnitude of effects for evaluation in the Biological Opinion (BO), some estimate of the chronic effects of cyanide on LAA listed species is needed. The approach taken here was adapted from the screening level assessment employed in the Biological Evaluation (BE) methodology (EPA 2006). According to the BE methodology, if chronic data for the listed species are not available the chronic threshold for the listed species, i.e. the "Assessment Effects Concentration" or EC_A is estimated using data from surrogate species. The chronic EC_A is intended to estimate the highest chemical concentration in water (for aquatic species) or food (for aquatic-dependent species) that would cause no adverse effect or would adversely affect an acceptably-small percentage of individuals within a specified species population. For chronic toxicity, the EC_A is based on the acute toxicity to the listed species (LC_{50}), and the Acute to Chronic Ratio (ACR) of surrogate species. The ACR, as defined in the national BE methodology, is calculated as follows:

$$ACR = \frac{SS\ LC_{50}}{SS\ NOEC} \qquad (1)$$

Where: SS LC_{50} is the LC_{50} for the surrogate species

SS NOEC is the No Observable Effects Concentration for the surrogate species

Chronic EC_A's are estimated using the following equation:

$$\text{Chronic } EC_A = \frac{LS\ LC_{50}}{ACR} \qquad (2)$$

Where: LS LC_{50} is the LC_{50} for the listed species

The ACR and chronic EC_A are graphically illustrated in Figures 1 and 2. The effects determination for the BE is based on a comparison of the chronic EC_A for a listed species and the Criterion Continuous Concentration (CCC) for cyanide, 5.2 ug CN/L. If the chronic EC_A is less than 5.2 ug CN/L the species is likely to be adversely affected and if the EC_A is greater than 5.2 ug CN/L the species is not likely to be adversely affected. The

type of effect and its severity (beyond likely versus not likely) were not part of the BE, but that information is needed for the BO to help characterize the magnitude of effects to individuals, populations and the species as a whole. As mentioned above, the BE method was adapted in order to estimate the magnitude of effect that would occur if the listed species was exposed to cyanide at the CCC.

Equations 1 and 2 can be combined by substituting equation 1 for the ACR term in equation 2:

$$\text{Chronic } EC_A = \frac{\text{LS } LC_{50}}{\frac{\text{SS } LC_{50}}{\text{SS NOEC}}}$$

Rearrange:

$$\text{Chronic } EC_A = \frac{\text{LS } LC_{50}}{\text{SS } LC_{50}} * \text{SS NOEC} \qquad (3)$$

Figure 3 illustrates how equation 3 can also be used to calculate the chronic EC_A. In this case the relative difference in sensitivity between listed species and surrogate species to acute exposures is used as a sensitivity adjustment factor to calculate the chronic EC_A for the listed species from the surrogate species NOEC.

Although, the EC_A term is defined as the "Assessment Effects Concentration" it is essentially the estimated "NOEC" for the listed species. Thus, the concentration of cyanide that causes a particular level of effect in surrogate species is used to estimate the concentration that causes the same level of effect in listed species. Based on this concept we rewrote equation 3 in a more general form:

$$\text{LS } EC_X = \frac{\text{LS } LC_{50}}{\text{SS } LC_{50}} * \text{SS } EC_X \qquad (4)$$

Where:

LS EC_X is the Effects Concentration for the listed species that elicits a response of magnitude X (replacing the chronic EC_A term), and;

SS EC_X is the Effects Concentration for the surrogate species that elicits a response of magnitude X (replacing the SS NOEC term).

With Equation 4, estimations are not limited to "NOEC" concentrations. Depending on the data available for the surrogate species, a range of effect concentrations (e.g. EC_{10}, EC_{20}, etc.) may be estimated. However, for the Biological Opinion, we are interested in estimating the magnitude of effect on listed species when exposed to cyanide at a specific

concentration, i.e. the CCC (5.2 ug CN/L). Equation 4 can be used to derive this estimate. Because we are interested in the magnitude of effect occurring at the CCC, we first set the LS EC_X equal to the 5.2 ug CN/L, then calculate the Effects Concentration for the surrogate species, SS EC_X, and finally estimate X (magnitude of effect) from the exposure – response relationship for the surrogate species:

First, set

$$LS\ EC_X = 5.2\ ug\ CN/L$$

Substitute in equation 4,

$$5.2\ ug\ CN/L = \frac{LS\ LC_{50}}{SS\ LC_{50}} * SS\ EC_X$$

Next, rearrange:

$$SS\ EC_X = \frac{SS\ LC_{50}}{LS\ LC_{50}} * 5.2\ ug\ CN/L \qquad (5)$$

Because the SS LC_{50} and LS LC_{50} are known (or estimated), setting the LS EC_X equal to the 5.2 ug CN/L allows for the calculation of SS EC_X. The SS EC_X is the effects concentration for the surrogate species that is equivalent to the effects concentration for the listed species at 5.2 ug CN/L, after adjusting for differences in sensitivity between the surrogate and listed species based on the ratio of acute toxicities, i.e. SS LC_{50}/LS LC_{50}.

For example, the LC_{50} for fathead minnow, a surrogate species, is 138 ug CN/L[1] and the estimated LC_{50} for the Maryland darter (*Etheostoma sellare*), a listed species, is 40 ug CN/L[2]. Based on these values fathead minnows would be 3.45 times less sensitive than Maryland darters and the SS EC_X would be 17.9 ug CN/L, that is, 3.45 times higher than the CCC (5.2 ug CN/L). In other words, fathead minnows would have to be exposed to a concentration 3.45 times higher than the CCC to experience an effect, equal in magnitude, to the effect on the Maryland darter exposed at the CCC.

Once the SS EC_X is calculated, the magnitude of effect (X) can be estimated using the chronic exposure-response curve for the surrogate species (Figure 4). In this illustration the chronic toxicity data for the fathead minnow was fitted to a log-linear regression model. The magnitude of effect, X, at the SS EC_x (17.9 ug CN/L) can be estimated using this

[1] Source: Table 4 of the Cyanide Biological Opinion
[2] Source: Table 4 of the Cyanide Biological Opinion, surrogate taxa - the genus *Etheostoma*.

model. For fathead minnow, an SS EC_x of 17.9 ug CN/L corresponds to an effect of about 54%. Therefore, the CCC (5.2 ug CN/L) would correspond to an EC_{54} for the Maryland darter, or a 54% effect concentration. Please note that this example is for illustration purposes only. Refer to the chronic effects section of the cyanide BO for detailed explanations of how the approach was implemented including, regression models and estimated effects levels. In addition, the section entitled *Population Responses to Reductions in Fecundity and Juvenile Survival* discusses linkages between individual and population-level effects.

This estimation approach relies on two underlying assumptions: (1) that relative differences in sensitivity between surrogate species and listed species to acute exposures are good approximations of the relative differences in sensitivity to chronic exposures and (2) that the slope of the exposure-response curves for surrogate and listed species are reasonably similar. The chronic effects section of the cyanide BO includes an assessment of method performance.

References

EPA [U.S. Environmental Protection Agency]. 2006 (*Draft*). Draft Framework for Conducting Biological Evaluations of Aquatic Life Criteria, Methods Manual.

Figure 1. Illustration of the Acute to Chronic Ratio (ACR) using acute and chronic data for fathead minnow.

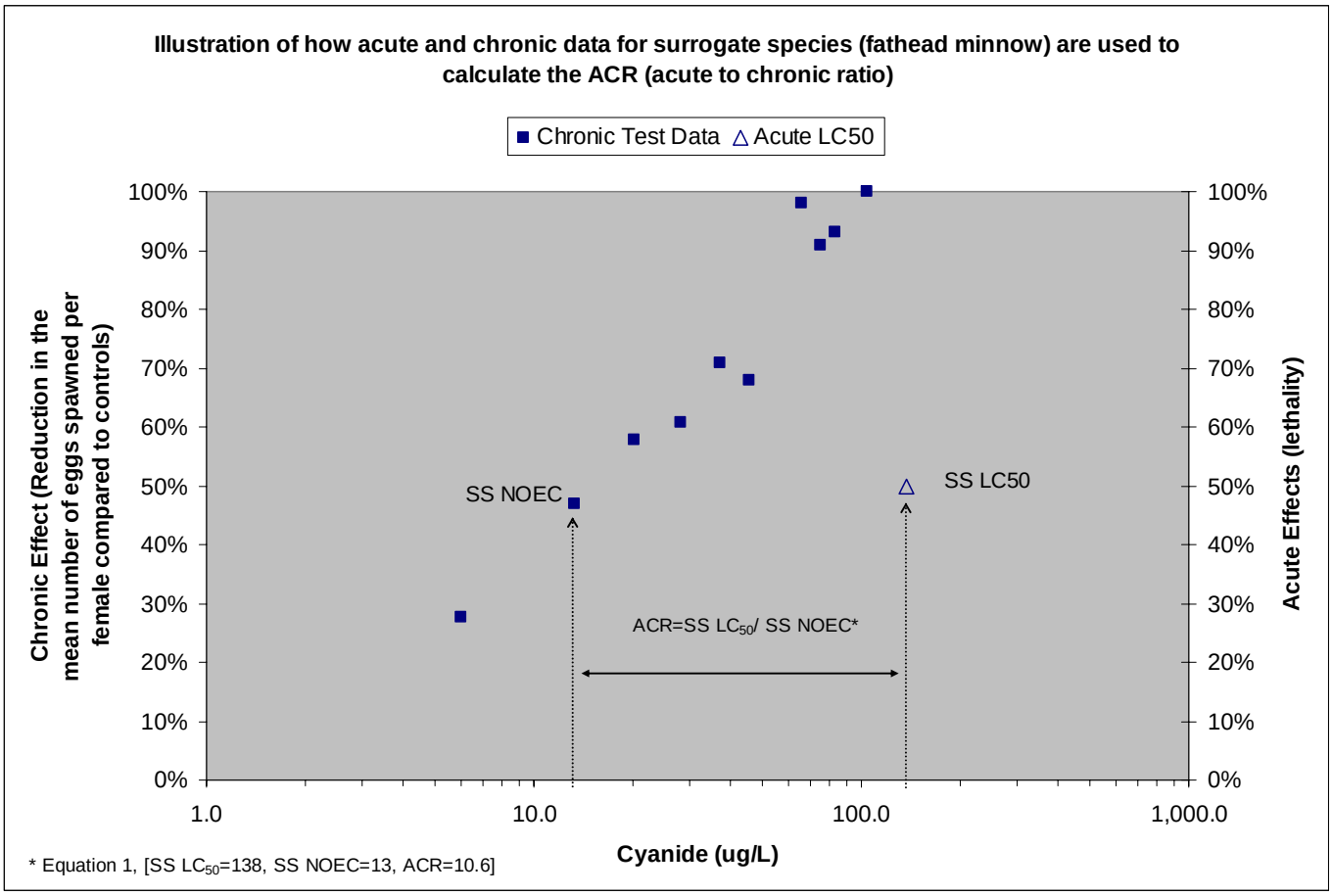

Figure 2.

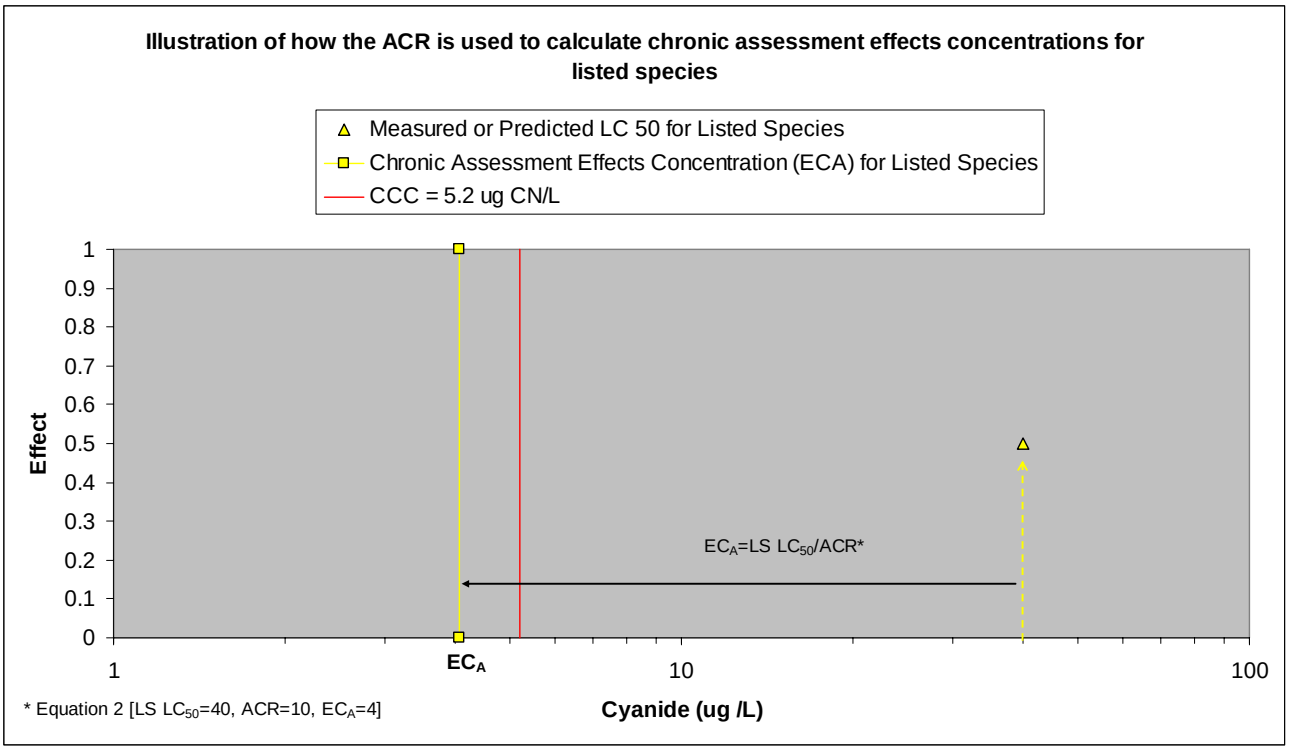
Illustration of how the ACR is used to calculate chronic assessment effects concentrations for listed species
Measured or Predicted LC 50 for Listed Species
Chronic Assessment Effects Concentration (ECA) for Listed Species
CCC = 5.2 ug CN/L
Effect
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
EC_A=LS LC_50/ACR*
1
EC_A
10
100
* Equation 2 [LS LC_50=40, ACR=10, EC_A=4]
Cyanide (ug /L)

Figure 3.

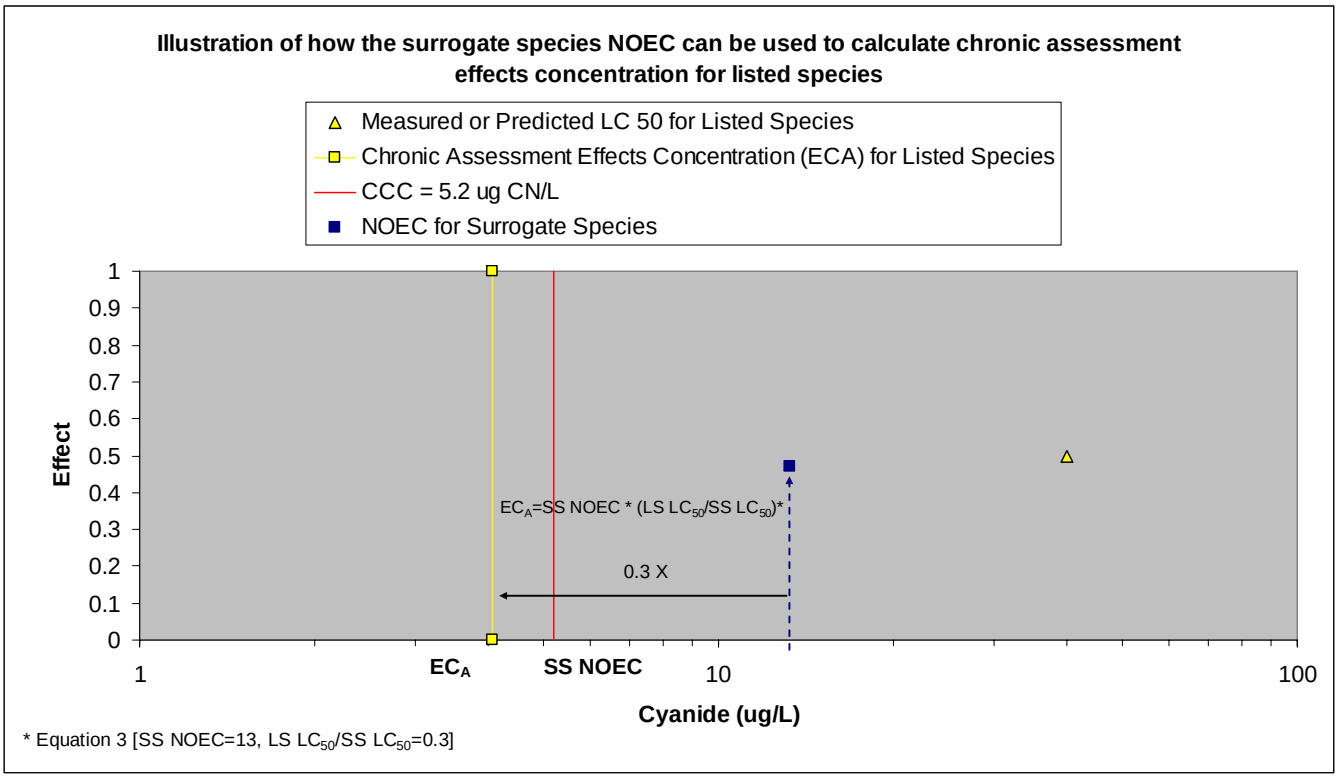
Illustration of how the surrogate species NOEC can be used to calculate chronic assessment effects concentration for listed species
Measured or Predicted LC 50 for Listed Species
Chronic Assessment Effects Concentration (ECA) for Listed Species
CCC = 5.2 ug CN/L
NOEC for Surrogate Species
Effect
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
ECA=SS NOEC * (LS LC50/SS LC50)*
0.3 X
1
ECA
SS NOEC
10
100
Cyanide (ug/L)
* Equation 3 [SS NOEC=13, LS LC50/SS LC50=0.3]

Figure 4.

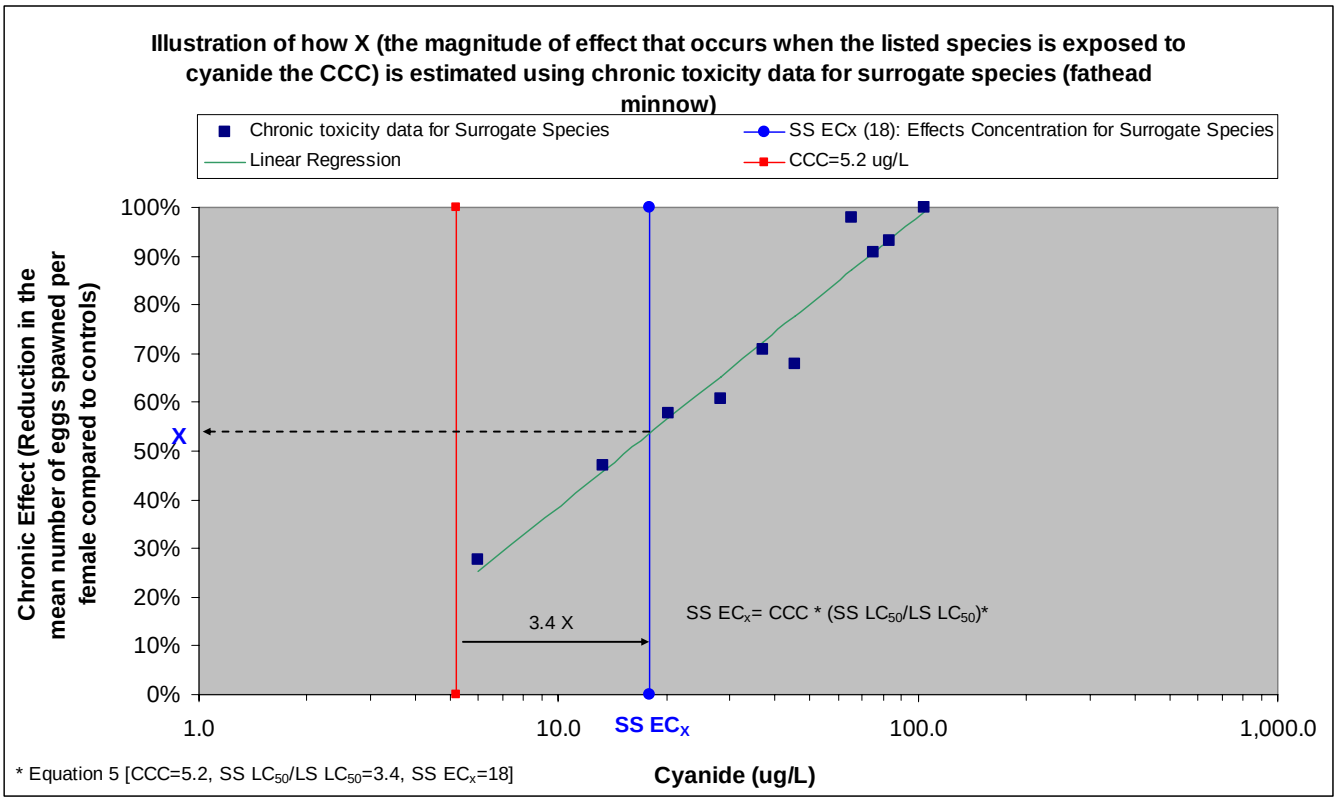
Illustration of how X (the magnitude of effect that occurs when the listed species is exposed to cyanide the CCC) is estimated using chronic toxicity data for surrogate species (fathead minnow)
Chronic toxicity data for Surrogate Species
SS ECx (18): Effects Concentration for Surrogate Species
Linear Regression
CCC=5.2 ug/L
Chronic Effect (Reduction in the mean number of eggs spawned per female compared to controls)
100%
90%
80%
70%
60%
X
50%
40%
30%
20%
10%
0%
3.4 X
SS ECx= CCC * (SS LC50/LS LC50)*
1.0
10.0
SS ECX
100.0
1,000.0
Cyanide (ug/L)
* Equation 5 [CCC=5.2, SS LC50/LS LC50=3.4, SS ECx=18]